

Histamine release from basophil leukocytes induced by microbial antigen preparations in patients with AIDS

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Type I allergy against some common microorganisms was investigated in 14 patients with AIDS and 11 human immunodeficiency virus (HIV) antibody-positive homosexual men, and in a control group consisting of 13 heterosexual men without HIV antibodies. Basophil histamine release technique was used as a sensitive method to detect type I allergy against *Candida albicans* (CA), Herpes simplex virus type I (HSV-I) and cytomegalovirus (CMV). Of the 14 AIDS patients 11 (78%) showed significant histamine release when stimulated with CA, and HSV-I caused release in 10 (71%), whereas no response was obtained by CMV. In the group of HIV antibody-positive men only one released histamine when stimulated with CA and HSV-I and this patient also had lymphadenopathia. In contrast to these results, no release of histamine was obtained in the control group consisting of 13 heterosexual men. The histamine release caused by CA and HSV-I is mediated by an immunological reaction, since the release was abolished and regained by removal from and refixation to the cell surface of the cell-bound immunoglobulins. These results suggest an involvement of type I allergy as a pathogenetic co-factor in some infections in AIDS, and allergic type I reactions to CA and HSV-I might be an indicator for the presence of manifest AIDS.

Key words: AIDS; basophil histamine release; *Candida albicans*; Herpes simplex virus type I; cytomegalovirus; type I allergy; infection.

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CLINICAL ASPECTS

Candida albicans and Herpes simplex virus type I infections can induce type I allergy in AIDS patients. Histamine and other mediators released from basophilocytes and mast cells by the type I allergic reaction could be an important factor in the course of the infections. Moreover, histamine is known to influence various immunological reactions. It might therefore be tempting to consider a treatment with anti-allergic or immunomodulating drugs of these patients.

Acquired immunodeficiency syndrome (AIDS) is characterized by multiple and diverse clinical and morphological manifestations and laboratory abnormalities (3). All patients have in common a dysfunction in T cell immunity and a high incidence of opportunistic protozoal, viral, bacterial or fungal infections (3). Immunological processes involving allergic type I reactions may also be important in the pathogenesis of AIDS. Thus, elevated serum IgE

concentration and eosinophil counts are found in some patients (1, 10) and in some patients an increased number of mast cells has been detected in the lymph node and the rectal mucosa (5, 11). Furthermore, a significantly higher frequency of allergic reactions to trimetoprim/sulfamethoxazole has been observed in AIDS patients compared with other immunodeficiency patients (6). In the present study we have investigated a possible involve-

ment of type I reaction in AIDS patients directed against the viruses, Herpes simplex virus type I (HSV-I) and cytomegalovirus (CMV) and against the fungus *Candida albicans* (CA).

MATERIAL AND METHODS

Patients

The investigation included 13 homosexual men and one heterosexual man, the latter having incurred (HIV) infection in Africa, all with AIDS according to the criteria of the Centers for Disease Control. The mean age was 36 years (range 28–54), and at the time of the investigation the duration of AIDS was 4 months (range 0–18). All patients showed antibodies against HIV, and revealed severe cellular immunodeficiency demonstrated by decreased number of T-helper (OKT4) cells and reduced lymphocyte response to mitogen and antigen in lymphoblast transformation test. Before the onset of AIDS two patients suffered from allergic rhinitis. Moreover, four AIDS patients showed adverse reaction to drugs with skin rash and fever. Three patients had Kaposi's sarcoma, two of them also had concomitant opportunistic infections, 12 had clinical symptoms and signs compatible with *Pneumocystis carinii* pneumonia, 11 had *Candida* stomatitis, of whom three also had *Candida* eosophagitis, five had Herpes simplex mucocutaneous infections, five showed cytomegalovirus in urine and/or in saliva and two patients had atypical mycobacteria in bone marrow or liver biopsy. The study also included 11 homosexual men with antibodies against HIV (HIV antibody-positive), but not fulfilling the criteria for AIDS. Three of these patients had lymphadenopathy as the only sign of HIV infection, and eight had no symptoms at all. 13 healthy age-matched heterosexual men without HIV antibodies were used as a control group.

Microbial antigen preparations and stimulators

Candida albicans (CA) was cultured on Sabouraud dextrose agar for 48 h at 37°C, and the

colonies were scraped off, washed twice and resuspended in saline (0.154 M NaCl) to a final concentration of 1 g wet weight/ml (2).

Herpes simplex virus type I (HSV-I). Human embryonic lung fibroblast cells were grown in minimal essential medium with 10% foetal calf serum. The fibroblasts were infected with five plaque-forming units per cell of HSV-I strain F, which was obtained from Professor B. Roizman, University of Chicago. Infection was made under standard conditions and the cells were maintained in minimal essential medium with 1% foetal calf serum (9). 20 h after the infection the monolayer cells were washed in phosphate buffered saline (PBS), scraped off into 10 ml PBS and pelleted by centrifugation. The cells were solubilized by sonication in a 0.020 M glycine; 0.0076 M Tris buffer (pH 8.6) with 1% Triton X-100. Triton X-100 was used to extract infected cell proteins more efficiently and to eliminate infectious virus particles by destruction of their envelope. The antigen preparation was clarified by ultracentrifugation at 100,000 *g* at 4°C. The concentration of total protein was 6–7 mg/ml.

Cytomegalovirus (CMV). Human embryonic lung cells grown in Eagle's minimal essential medium with 2% calf serum were infected with cytomegalovirus strain Ad 169, 1 PFU (plaque-forming unit) per cell. At the time of maximal cytopathic effect (about 6 days after infection), the culture was frozen and thawed twice and centrifuged at 1000 *g* for 10 min to spin down cellular debris. The supernatant fluid, containing approx. 10 > PFU/ml was used as antigen.

Rabbit antihuman IgE. (ε-chain 400,000 IE/ml) was obtained from Behringwerke, West Germany and Calcium-ionophore A 23187 from Calbiochem AG, Switzerland. All preparations were kept in small aliquots at –20°C.

Preparation of leukocyte suspension

Blood was drawn by venipuncture, anticoagulated by EDTA in a final concentration of 0.01

M and mixed with a double volume of 0.9% NaCl. Mononuclear cells with approx. 2% basophils were isolated by the Ficoll-Hypaque gradient method, as previously described (13). To prevent histamine release by activation of complement as a source of error, the cells were washed twice (300 g for 10 min) in Tris-AMC (albumin, magnesium, calcium) buffer containing Tris (hydroxymethyl) amino methane 25 mM at pH 7.4, NaCl 0.12 M, KCl 5 mM, CaCl_2 0.6 mM, MgCl_2 1.1 mM, human serum albumin 0.3 mg/ml, and glucose 1 mg/ml, and finally resuspended in this buffer.

Removal of immunoglobulin from basophils

The acidification procedure of Stahl Skov et al. (12) was used for removal of Ig from the surface of leukocytes obtained from 50 ml whole blood. The leukocyte suspension was adjusted to pH 3.8 by 1 M HCl and left at 4°C for 5 min. After centrifugation at 700 g for 5 min, the cell pellet containing cells deprived of Ig was separated from the supernatant containing the removed Ig. The supernatant was readjusted to pH 7.4 with 5M NaOH and was freeze-dried, dialysed against water and resuspended in 0.5 ml Tris-AMC (supernatant preparation), for fixation of Ig to Ig-deprived cells.

Fixation of removed Ig to basophils

The cell pellet of acid-treated cells from 10 ml whole blood was incubated for 1 h at 37°C with 0.5 ml supernatant preparation for fixation of Ig to the Ig-deprived cells, whereafter the cells were washed and resuspended in 8 ml Tris-AMC pH 7.4.

Basophil histamine release

90 µl leukocyte suspension containing 700–1000 basophils was incubated in plastic tubes at 37°C for 40 min with 10 µl of the antigen preparations CA, HSV-I and CMV used in final dilutions of 10^{-13} to 10^{-4} , 10^{-11} to 10^{-3} and in 10^{-8} to 10^{-5} , respectively. Furthermore, other samples were challenged with anti-IgE in final dilutions of 10^{-11} to 10^{-3} , and with calcium-ionophore A 23187 in a

final concentration of 20 nM to 1.28 µM. For determination of spontaneous histamine release and total histamine content the addition of releasing agent was omitted for some cell samples, and for the latter purpose the cells were left at 4°C. The reaction was terminated by addition of 3 ml ice-cold Tris-AMC. The cell samples were thereafter centrifuged at 2000 g for 10 min. Besides liberated histamine, the supernatant contains proteins and other substances, which might interfere with the histamine analysis. The supernatants were therefore discarded, and residual histamine was determined in the cell sediment (13). The release was expressed as a percentage of the total histamine content of the sample. The spontaneous histamine release amounted to up to 10%, therefore only histamine release exceeding 10% was considered significant. Counting of lymphocytes, OKT4 cells, eosinophils and measurement of plasma IgE was performed according to the standard routine methods.

Statistics. The rank sum test was used (4) and with significance level at 5%.

RESULTS

The histamine release induced by antigen preparations of *Candida albicans* (CA), Herpes simplex virus type I (HSV-I) and cytomegalovirus (CMV) was examined *in vitro* by the basophil histamine release test in AIDS patients, HIV antibody-positive men, and in controls. Of 14 AIDS patients 11 responded to CA with a significant histamine release (Table 1). The maximal release of histamine induced by CA was observed with final dilutions of 10^{-11} to 10^{-5} , and the mean was 22% ranging from 12–34%. Ten of the AIDS patients responded to HSV-I, and the maximal release of histamine obtained in final dilutions of 10^{-9} to 10^{-3} amounted to 18% (12–24%) (Table 1). Fig. 1 shows representative curves for histamine release induced by CA and HSV-I. However, no histamine release was found when cells from AIDS patients were challenged with CMV antigen. (Table 1).

To verify an Ig-mediated reaction in histamine release caused by CA and HSV-I, Ig was removed from the basophilocytes by the acid-

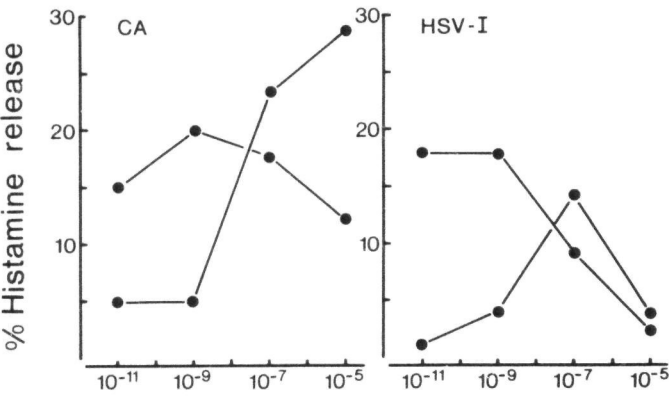


Fig. 1. Histamine release in leukocyte suspensions from AIDS patients caused by *Candida albicans* (CA) and Herpes simplex virus type I (HSV-I). Final dilutions are given for the antigen preparations. Two representative curves of each are given.

ification procedure prior to incubation with the antigen preparations. Fig. 2 shows that removal of functional IgE, demonstrated by the absence of response to anti-IgE, abolished the response to CA and HSV-I. Furthermore, refixation of the removed Ig to the Ig-deprived cells reestablished both cell-bound IgE and the histamine release induced by the antigen preparations (Fig. 2). An Ig-mediated reaction was further substantiated by a similar transfer of the Ig to acid-treated cells from a control person instead of from the patients. After fixation of patient Ig, the control cells were able to release histamine by challenge with CA and HSV-I (Fig. 3). That the fixation involves binding of IgE to the cell surface was shown by a positive response to anti-IgE (Fig. 3).

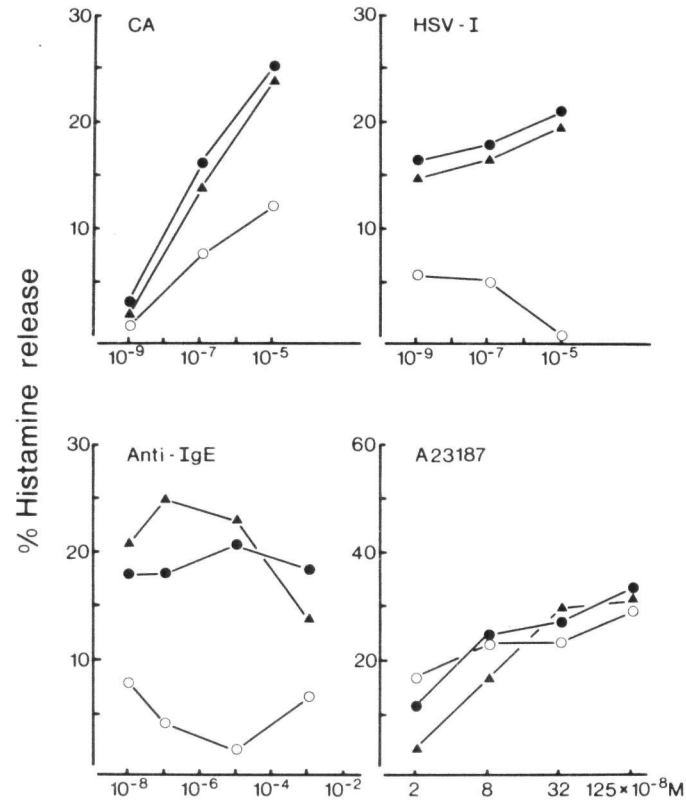


Fig. 2. Histamine release in leukocyte suspension from AIDS patients induced by CA, HSV-I, anti-IgE and A 23187. Intact cells (●—●), acid-treated cells deprived of Ig (○—○) and with addition of the deprived Ig (▲—▲).

Of the 11 patients in the HIV antibody-positive group only one showed a positive response to CA and HSV-I, and this patient had lymphadenopathy (Table 1). None of these patients released histamine when challenged with CMV. Of the 13 control persons none of

Table 1

Histamine release from basophilocytes stimulated by anti-IgE, *Candida albicans*, Herpes simplex virus type I, cytomegalovirus and calcium-ionophore A 23187. Significant histamine release means release above 10%. The mean maximal histamine release is given independent of the antigen/stimulator dilution, and the range is given in parenthesis

Subject		Stimulator				
		anti-IgE	CA	HSV-I	CMV	A23187
AIDS patients 14	patients with signifcant release	12	11	10	0	14
	mean of maximal release	21(12-34)	22(12-34)	18(12-24)	< 10	32
HIV antibody positive 11	patients with significant release	8	1	1	0	11
	mean of maximal release	22(13-33)	12	14	0	29(13-40)
Control group 13	persons with significant release	10	0	0	0	13
	mean of maximal release	21(13-38)				36(22-52)

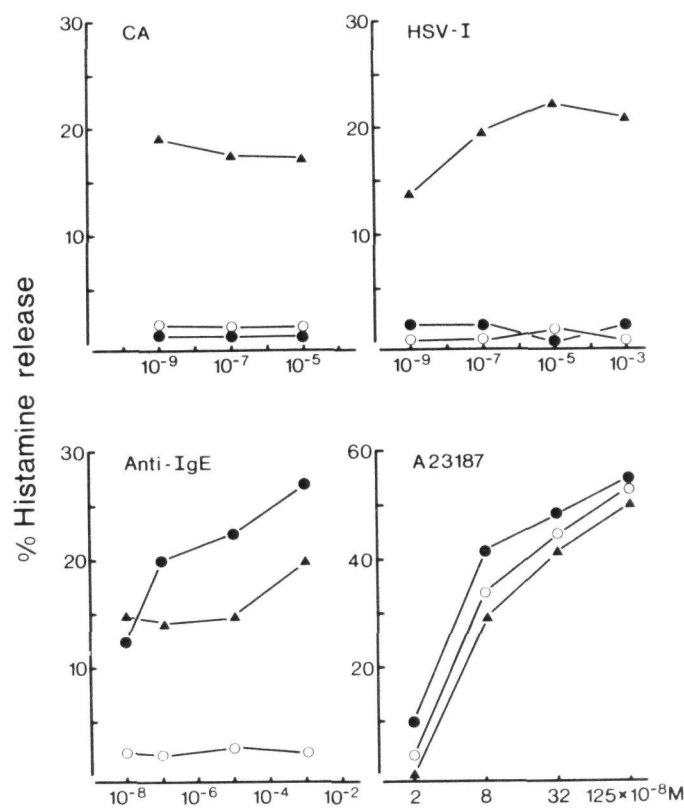


Fig. 3. Histamine release in leukocyte suspensions from normal individuals caused by CA, HSV-I, anti-IgE and A 23187. Intact cells (●—●), acid-treated cells deprived of own Ig (○—○), and with addition of Ig from AIDS patients (▲—▲).

them responded to any of the microbial antigens with histamine release (Table 1).

To differentiate between cellular responders and non-responders we used anti-IgE as the classical tool. Two of the AIDS patients were identified as non-responders, since they did not release histamine by anti-IgE. These two patients showed no release, either by CA, HSV-I or CMV.

Three of the HIV antibody-positive patients

and four control persons were non-responders, and the one patient who responded to CA and HSV-I was found to be a cellular responder.

The basic cellular function was elucidated by the non-immunological histamine release response to calcium-ionophore A 23187, which was used in a concentration range from 20 nM to 1.28 μ M. All patients and controls showed histamine release when stimulated with calcium-ionophore, and the test revealed no significant difference between the AIDS patients, the HIV antibody-positive men and the control group (Table 1), indicating that the cellular function was not changed in AIDS patients.

Of the five AIDS patients showing mucocutaneous Herpes simplex infections four released histamine when stimulated by HSV-I antigen. All AIDS patients except one had IgG antibodies against HSV. Apart from the patients who were non-responders, eight of nine patients with *Candida* stomatitis and/or eosophagitis showed histamine release when challenged with CA.

Table 2 shows the mean and range of IgE in serum, the number of eosinophils, OKT4 (T-helper) positive cells and the total counts of lymphocytes. Plasma IgE and number of eosinophils showed no significant difference between the groups. However, four patients with AIDS and two HIV antibody-positive men had elevated values of IgE. One of these AIDS patients and one HIV antibody-positive patient had since childhood suffered from rhinitis and atopic dermatitis, respectively. None of these patients with elevated IgE had a family history of atopy. Eosinophilia was seen in four AIDS patients and one HIV antibody-positive pa-

Table 2
Laboratory parameters. Mean value and range

		Plasma IgE I.E./ml	Eosinophils × 10 ³ /ml	OKT4 × 10 ⁶ /ml	Lymphocytes × 10 ⁶ /ml
AIDS patients	mean	163	289	0.12*	1.3*
	range	6-665	0-1606	0.02-0.40	0.2-4.5
HIV antibody positive	mean	82	223	0.81*	3.2*
	range	5-555	63-431	0.39-1.22	1.6-5.0
Normal values		3-263	<350	>0.5	0.5-4.6

* $P < 0.01$ between AIDS patients and HIV-positive patients.

tient. The number of OKT4 positive cells and total lymphocytes were significantly lower in patients with AIDS than in HIV antibody-positive patients (Table 2). All AIDS patients with elevated IgE and/or eosinophilia had at least two opportunistic infections.

DISCUSSION

Patients with AIDS show a high incidence of opportunistic protozoal, viral, bacterial and fungal infection (3), and infection with CMV or Epstein-Barr virus are associated with a high frequency of hypersensitivity reactions, such as cutaneous manifestations to certain antimicrobial agents used (3). Elevated serum IgE contractions and eosinophil counts are found in some patients with AIDS (1, 10). These observations could indicate that AIDS patients may develop type I allergy against various microbial agents.

Infections caused by *Candida albicans*, Herpes simplex virus and cytomegalovirus are very common in AIDS patients, and these microorganisms exist in many healthy individuals. Type I allergy against these microbial antigens was investigated in the present study, using the basophil histamine release technique (13) as a sensitive and specific way to detect type I allergy. CA and HSV-I induced histamine release in approximately 75% of AIDS patients. Only one patient in the group of HIV antibody-positive men released histamine by these antigens and this patient had lymphadenopathia. None of the control group released histamine when challenged with these antigens. CMV was found not to release histamine in any of the investigated groups, although 13 of 14 patients had positive CMV serology and six showed CMV viremic infection. Therefore, AIDS patients do not appear to develop type I allergy against CMV.

The histamine release caused by CA and HSV-I was found to be mediated by an immunological reaction, since the release was abolished by removal of Ig from the cell surface and regained by fixation of the Ig to the Ig-deprived cells. It seems very likely that the histamine release is caused by an IgE-mediated reaction,

since, by removal of Ig, functional IgE was also removed as demonstrated by the absence of response to anti-IgE (Fig. 2). Furthermore, by fixation of the Ig to the Ig-deprived cells the response to anti-IgE was reestablished (Figs. 2 and 3). However, it cannot be deduced from these experiments whether IgE is responsible or whether other immunoglobulins are involved in the histamine release caused by the microbial antigen preparations. AIDS patients showed a higher level of serum-IgE than controls, although this trend was not significant (Table 2). A contribution of cellular hyperreactivity as part of the histamine release by CA and HSV-I does not seem to be the case, since a change in the basic cell function compared with cells from normal individuals was not obtained, as estimated by the response to calcium-ionophore acting intracellularly (8). From type I allergic patients it is known that some of the persons will not show basophil histamine release by challenge with specific antigen or anti-IgE (7). In analogy some of the AIDS patients did not respond to anti-IgE, and these patients also showed no histamine release by the microbial antigen preparations. The reason could be lack of specific IgE in serum and/or on the cell surface, or a defect in some subcellular event leading to histamine release. If so, these events should precede the calcium-dependent steps, since a normal response was obtained by the calcium-ionophore A 23187. Therefore we cannot conclude whether these patients have IgE directed against microbial antigens.

The histamine release by CA was found to be well correlated with the clinical manifestations of candidiasis, since eight of nine AIDS patients with *Candida* stomatitis and/or eosophagitis showed histamine response. A similar correlation for HSV-I cannot be deduced, since only half of the patients who responded to HSV-I with histamine release showed mucocutaneous Herpes simplex infections, while in the other patients the possibility of subclinical reactivation of the infections may exist, since all AIDS patients except one showed IgG antibodies against HSV.

Our results indicate that patients with AIDS develop an allergic type I reaction to certain

microbial antigens, since these antigens can induce histamine release from the basophils of the patients and since this response was abolished and regained by removal and refixation of cell-bound immunoglobulin. This allergic reaction might be a pathogenic co-factor in some infections in AIDS, and allergic type I reaction to CA and HSV-I might be an indicator of the presence of manifest AIDS.

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